

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: ) Confirmation No. 1770  
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 Rey-Yuh Wu et al. )  
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 Application No.: 10/749,323 ) Group Art Unit: 1642  
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 Filed: December 31, 2003 ) Examiner: Fetterolf, Brandon J.  
 )  
 For: ASTRAGALUS RADIX AND CODONOPSIS )  
 PILOSULAE RADIX MIXED EXTRACT FOR )  
 INHIBITING CARCINOGENESIS AND METASTASIS) Docket No: 03-1119

Declaration under Rule 1.132

I, Bor-Shiun Chen, declare:

1. I am a named co-inventor on the subject application;
2. I performed the experiments described below. These experiments provide the result that the Astragalus radix and Codonopsis pilosulae radix mixed extract prepared according to the subject invention has a significant effect on inhibiting colon carcinogenesis and metastasis. To the contrary, Shenqi Fuzheng as described by Zhou Kexin et al. (Chinese Journal of Combined Traditional and Modern Medicine 1999; 19:11-13) fails to show a significant effect on inhibiting colon carcinogenesis and metastasis.
3. As described in Appendix 1, after intra-splenic implantation with colon cancer cell line CT-26 into BALB/c mice, the mice were treated with Astragalus radix and Codonopsis pilosulae radix mixed extract having the same weight ratios of Astragalus radix and Codonopsis pilosulae radix (with a total dosage of 0.2 and 0.6 g/kg) and Shenqi Fuzheng (with a dosage of 10 mL/kg). On day 14, the liver nodules were estimated. Shenqi Fuzheng was found to fail to inhibit colon carcinogenesis and metastasis significantly. On the other hand, 0.6 g/kg of the Astragalus radix and Codonopsis pilosulae radix mixed extracts inhibit colon carcinogenesis and metastasis most significantly ( $P < 0.01$ ). The Astragalus radix and Codonopsis pilosulae radix mixed extracts with a dosage of 0.2 g/kg also effect the inhibition of colon carcinogenesis and metastasis ( $P < 0.05$ ).

I hereby declare that all statements made herein of my own knowledge are true and that all

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statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by a fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature Bor-Shiun Chen Date May 17, 2006  
Bor-Shiun Chen



## Appendix 1

## **EFFECTS OF THE ASTRAGALUS RADIX AND CODONOPSIS PILOSULAE RADIX MIXED EXTRACT AND SHENQI FUZHENG**

The effects of inhibiting carcinogenesis and metastasis of the Astragalus radix and Codonopsis pilosulae radix mixed extract with a total dosage of 0.2 or 0.6 g/kg and Shenqi Fuzheng were assayed.

The tested pharmaceutical compositions include:

- (1) SGHF: Astragalus radix and Codonopsis pilosulae radix mixed extract prepared with water extraction followed by ultra-filtration; wherein the weight ratio of Astragalus radix:Codonopsis pilosulae radix is 1:1;
- (2) SGFD: Astragalus radix and Codonopsis pilosulae radix mixed extract prepared with water extraction, filtration followed by dialyzing; wherein the weight ratio of Astragalus radix:Codonopsis pilosulae radix is 1:1; and
- (3) CP-10ml: Shenqi Fuzheng with a dosage of 10 mL/kg.

Male BALB/c mice, six weeks old, were purchased from the National Laboratory Animal Center in Taiwan. Diet and tap water were supplied *ad libitum* throughout the study period. The mice were divided into eight groups, with each group containing eight mice and each cage containing four mice. The mice were subjected to intra-splenic implantation with colon cancer cell line CT-26 ( $2 \times 10^4$  cells/mouse) when they were eight weeks old. The mice were weighted and anesthetized by receiving adequate dose of sodium pentobarbital (Somnotol, Canada MTCPHAR, 10  $\mu$ L/g, 6.5 mg/ml) based on the body weight. A dose of 100  $\mu$ L CT-26 cells ( $2 \times 10^5$  cells/ml) were injected into the mice's spleens and then the mice were sutured with clips. The animal was protected from hypothermia until the animal was recovery. SGHF, SGFD and CP-10ml were administrated to the mice. The fourteenth day after implantation of CT-26, the mice were sacrificed, autopsied and the nodules on the livers were counted and recorded. The results are shown in FIG.1. Shenqi Fuzheng was found to fail to inhibit colon carcinogenesis and metastasis significantly. On the other hand, 0.6 g/kg of the Astragalus radix and Codonopsis pilosulae radix mixed extracts prepared with ultra-filtration and filtration followed by dialyzing inhibit colon carcinogenesis and metastasis most significantly ( $P < 0.01$ ). The Astragalus radix and Codonopsis pilosulae radix mixed extracts with a dosage of 0.2 g/kg also effect the inhibition of colon carcinogenesis and metastasis ( $P < 0.05$ ).

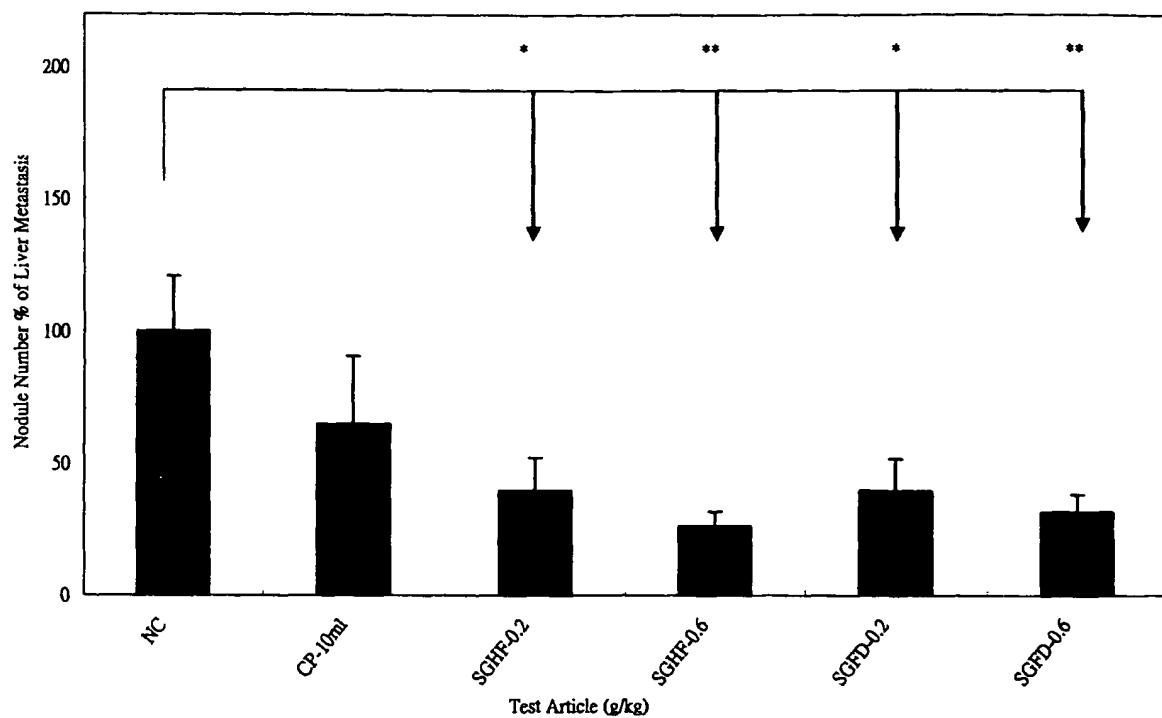


FIG.1: An anti-metastatic efficacy test was conducted to compare the efficacy among the test articles in CT-26 tumor-bearing BALB/c mice model by oral gavage. Forty-eight mice were used and randomized into 6 groups, and each group consisted of 8 male mice. The treated mice were administered with various test articles, containing of Astragalus radix and Codonopsis pilosulae radix mixed extract having the same weight ratios of Astragalus radix and Codonopsis pilosulae radix (SGHF, SGFD with a dosage of 0.2 and 0.6 g/kg) and Shenqi Fuzheng (CP with a dosage of 10 mL/kg) respectively. The mice were subjected to intra-splenic implantation with colon cancer cell line CT-26 ( $2 \times 10^4$  cells/mouse) when they were eight weeks old. The fourteenth day after implantation of CT-26, the mice were sacrificed, autopsied and the nodules on the livers were counted and recorded. \* represents significant difference of  $P < 0.05$  compared with NC (control). \*\* represents significant difference of  $P < 0.01$  compared with NC (control).